stacking is reversible and force dependent. We measure a stacking energy of $17k_BT$ and an unstacking distance that indicates the full exposure of the linker DNA. The time traces at constant force between 3.5 pN and 6.0 pN show that multiple nucleosome unstacking and restacking events take place simultaneously and non-cooperatively. The salt dependence of unstacking suggests competition between monovalent ions and divalent ions. These experiments provide the first single molecule data on nucleosome stacking and define a dynamic framework for chromatin organization in higher order structures.

2461-Pos
DNA Methylation Induces a More Compact and Rigid Nucleosome Structure
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Cytosine methylation in CpG dinucleotides is an important epigenetic modification in eukaryotes with roles in regulating a variety of genome transactions. There have been many studies of DNA methyltransferases and methyl-CpG binding proteins elucidating their roles in various genome activities. However, less is known about how methylated CpGs directly affect nucleosome structure. We implemented a single molecule FRET coupled with anisotropy that can simultaneously measure dynamic distance changes and flexibility of the two ends of a nucleosomal DNA. Using the method, we monitored effects of DNA methylation on the structure of mononucleosomes. In the absence of methylation, most nucleosomes displayed two low FRET states (FRET efficiency <0.5). When treated with a CpG methyltansferase, we observed a >40-fold increase in the number of nucleosomes that made excursions to a high FRET state (FRET efficiency >0.7). Moreover, based on the anisotropy measurements, a stronger association between the DNA ends and the histone octamer was observed from the nucleosomes in the high FRET state. The increased FRET and anisotropy after DNA methylation strongly suggest a more compact and rigid nucleosomal structure and provide a basic biophysical understanding of how DNA methylation may contribute to the formation of a repressive and transcriptionally inactive chromatin structure.

2462-Pos
Binding of the CHD4 PHD2 Finger to Histone H3 is Modulated by Covalent Modifications
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CHD4 (chromodomain helicase DNA-binding protein 4) ATPase is a major subunit of the repressive NuRD (nucleosome remodeling and deacetylase) complex, which is involved in transcriptional regulation and development. CHD4 contains two plant homeodomain (PHD) fingers of unknown function. Here we show that the second PHD finger (PHD2) of CHD4 recognizes the amino-terminus of histone H3 and that this interaction is facilitated by acetylation or methylation of Lys9 (H3K9ac and H3K9me, respectively) but is inhibited by methylation of Lys4 (H3K4me) or acetylation of Ala1 (H3A1ac). An 18 μM binding affinity toward unmodified H3 rises to 0.6 μM for H3K9ac and to 0.9 μM for H3K9me3, while dropping to 2.0 mM for H3K4me3, as measured by tryptophan fluorescence and NMR. A peptide library screen further shows that phosphorylation of Thr3, Thr6 or Ser10 abolishes this interaction. A model of the PHD2-H3 complex, generated using a combination of NMR, data-driven docking and mutagenesis data, reveals an elongated site on the PHD2 surface where the H3 peptide is bound. Together our findings suggest that the PHD2 finger plays a role in targeting of the CHD4/NuRD complex to chromatin.

2463-Pos
Theoretical Model of HP1-Induced Heterochromatin Formation
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In the cell nucleus, certain regions of the genome form a condensed fiber structure called heterochromatin, while the rest is more loosely packed into euchromatin. Heterochromatin protein 1 (HP1) accumulates in the condensed heterochromatin regions, binding to nucleosomes that are methylated at lysine-9 of histone 3. We perform simulations on chromatin fibers to find their idealized fiber structure for different linker lengths, incorporating the local geometry of the nucleosomes. From these fiber structures, we look at optimal geometric arrangements to maximize connectivity between HP1 binding sites on adjacent fibers and study this connectivity in a three-dimensional lattice. Since HP1 is known to dimerize and is purported to form a network structure with other factors to stabilize the condensed state, we look at stability of HP1 binding to this three-dimensional lattice. In particular, we address whether these HP1 interactions and the connectivity are sufficient to generate a phase separation, whereby the HP1 accumulates in the condensed heterochromatin but is removed from binding in the euchromatin regions. We look at this phenomenon for different chromatin fiber configurations to address the role of local nucleosome arrangement on global chromatin condensation.

2464-Pos
Applications of VDNA from Basic Geometry to Chromatin Folding
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We recently introduced VDNA(1), a plugin for VMD, that allows users to construct arbitrarily complex models of double stranded DNA. VDNA is preinstalled in VMD 1.8.7 and has a number of predefined mathematical expressions that allow for investigation of the complex relations between the DNA base pair step parameters (Tilt, Roll, Twist, Shift, Slide, Rise) and spatial conformation. VDNA is capable of producing static images, ensembles and trajectories. Using VDNA we demonstrate some of the geometric relations between the DNA step parameters and Cartesian coordinates that do not readily yield to intuition. We also consider a number of biologically motivated models including: a model of thermal fluctuations in linear DNA, comparison of a Torsion Helix and a Shear Helix model of the nucleosome(2), and models for regular and irregularly folded chromatin. Finally we demonstrate how VDNA can be used to convert nucleosome footprints from a chromatin map into a three dimensional chromatin fold. Such folding provides an immediate answer as to whether or not the footprints yield a conformation that is sterically allowed or if extra-nucleosomal proteins, non-canonical nucleosome conformations or thermal effects should also be considered. The most up to date version of VDNA is available from http://dna.ccs.tulane.edu/vdna.
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2465-Pos
Regulation of Nucleosome Conformational Dynamics by Post-Translational Histone Modifications Studied with Single-Pair FRET
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Nucleosomes form the basic unit of DNA compaction in eukaryotes. Not only do they condense the DNA, nucleosomes also play a crucial role in gene regulation: they modulate access to nucleosomal DNA for DNA-processing proteins. DNA within the nucleosome is made accessible via a combination of conformational changes caused by spontaneous fluctuations (DNA breathing), and by ATP-dependent remodeling enzymes. Both mechanisms are regulated by specific post-translational modifications to the nucleosome histones. Histone acetylation at H3K56, for example, has been shown to induce increased gene expression *in vivo*.
To characterize the effects of specific histone modifications on conformational dynamics of *individual nucleosomes*, we perform single-pair FRET (spFRET) measurements. We reconstitute nucleosomes from DNA labeled with a FRET pair and either modified or unmodified histones. The modified histones are obtained using a novel genetic code expansion technique that allows for genetically defined incorporation of modified amino acids. By placing FRET labels at different positions on the nucleosomal DNA, we show that transient DNA unwrapping occurs progressively from both nucleosome ends for up to at least 40 basepairs.
We follow DNA breathing dynamics of individual nucleosomes by combining spFRET and Alternating Laser Excitation (ALEX) with TIRF microscopy on immobilized nucleosomes. Alternatively, we combine spFRET and ALEX with gel electrophoresis and Fluorescence Correlation Spectroscopy (FCS) to diffusing nucleosomes. We show that a single acetylation at H3K56 increases DNA breathing of the first ~20 basepairs at least 2-fold. Furthermore, the initial state shifts to a more unwrapped conformation. Comparison with a simple model that assumes unwrapping of the DNA in ten-basepair steps indicates that acetylation at H3K56 causes the first DNA-histone contact point to break. Using these techniques we aim to further quantify epigenetic changes in chromatin at the single-molecule level.